

# Metabolic risk markers in an overweight and normal weight population with oversampling of carriers of the IRS-1 972Arg-variant

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## Metabolic risk markers in an overweight and normal weight population with oversampling of carriers of the IRS-1 972Arg-variant

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### Abstract

The relationship between the *Gly972Arg* polymorphism in the insulin receptor substrate-1 (IRS-1) gene and metabolic risk markers is not clear, possibly due to small sample sizes. Modification by body mass index (BMI) has also been suggested. Our aim was therefore to quantify the association of this 972Arg-variant with insulin, glucose and lipid levels in overweight and non-overweight subjects with oversampling of subjects with the 972Arg-variant. We first genotyped 3684 subjects selected from a large population-based cohort ( $n \sim 23,000$ ) according to BMI (26–40 or 18–24 kg/m<sup>2</sup>). Next, we examined 600 of these subjects for fasting metabolic risk markers according to BMI-group and genotype. Subjects with the 972Arg-variant had significantly higher insulin concentrations (4.09 pmol/l or 9.6%,  $P = 0.024$ ) and lower triglyceride levels (0.13 mmol/l or 11%,  $P = 0.001$ ) compared with non-carriers when adjusted for age, sex, waist-to-hip ratio, BMI, alcohol consumption, physical activity and cigarette smoking. These associations were more pronounced in the high BMI-group, although the interactions were not statistically significant. Our large population-based sample shows that the IRS-1 *Gly972Arg* polymorphism relates to higher fasting insulin levels and lower triglyceride levels. The impact of this genotype and its modification by overweight may be smaller than suggested previously. © 2003 Elsevier Ireland Ltd. All rights reserved.

**Keywords:** Obesity; IRS-1; *Gly972Arg*; Insulin resistance; Serum lipids; Genetic associations

### 1. Introduction

Insulin receptor substrate-1 (IRS-1) occupies a key position in the insulin signalling pathway [1]. After binding of insulin to the  $\alpha$ -subunit, the  $\beta$ -subunit of the insulin receptor undergoes autophosphorylation, and in turn, phosphorylates other endogenous protein substrates in the cascade of insulin action. As IRS-1 is the first substrate in this cascade, an impaired IRS-1 function may result in a defect in insulin signalling.

Several polymorphisms in the IRS-1 gene, located on chromosome 2q36, have been found [2]. The association between the so-called *Gly972Arg* polymorphism and phenotypes in humans has been studied most extensively, probably because the prevalence of this genotype is relatively high (approximately 10%) [3–9]. We recently reported the results

of a meta-analysis of 27 studies on the association between this variant and the risk for type 2 diabetes, showing a 25% increased risk [3]. However, considerable heterogeneity was observed.

Results of the impact on metabolic risk factors such as insulin resistance are also not always consistent. Almind [4] found a higher proportion of carriers among type 2 diabetic patients compared to controls, but diabetic carriers had lower fasting insulin concentrations as compared with non-carriers. Others did not observe differences in fasting insulin concentrations or insulin sensitivity between type 2 diabetic patients with and without the 972Arg-variant [8,9]. On the other hand, the proportion of carriers was higher in type 2 diabetic patients with either insulin resistance or dyslipidaemia [10]. The frequency of the 972Arg-variant was also significantly higher among Italian patients with coronary heart disease (CHD) than among controls [6]. In this study, no associations between insulin or glucose concentrations with genotype were observed, but independent of CHD, total cholesterol concentrations were higher in carriers than in

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non-carriers. In contrast, a British study including patients with diabetes or CHD reported lower total cholesterol and triglyceride concentrations in carriers compared with non-carriers [9].

The relationship between the *Gly972Arg* polymorphism of the IRS-1 gene and cardiovascular risk parameters may depend on body weight. This is suggested by the results of Clausen et al. [7], who observed an association between the polymorphism and increased fasting glucose, triglyceride, tissue plasminogen activator concentrations, and decreased insulin sensitivity in obese but not in non-obese subjects. Also Baroni et al. [5] observed an association between the IRS-1 variant and metabolic cardiovascular risk parameters in obese, but not in non-obese healthy subjects. However, the number of carriers of the IRS-1 variant in previous studies was generally small, which probably explains a large part of the inconsistent findings.

The aim of the present study is therefore to quantify the interrelationships between the *Gly972Arg* IRS-1 polymorphism and obesity on insulin, glucose and lipid levels in a large population-based study with oversampling of carriers of the polymorphism.

## 2. Subjects and methods

### 2.1. Subjects

Subjects were selected from participants of the Cardiovascular Disease Risk Factor Monitoring Project and the Monitoring Project on Risk Factors for Chronic Disease (MORGEN study) in the Maastricht area, in the south of The Netherlands. A detailed description of these studies has been published elsewhere [11,12]. The first examination, which included height and weight measurements and collecting of non-fasting blood samples, was carried out between 1987 and 1997. Buffy coats were isolated and stored at  $-20^{\circ}\text{C}$ . A re-approach including a questionnaire on body weight took place in 1998.

For the present study, we selected two groups of Caucasian subjects from whom buffy coats were available. The first group of subjects had a body mass index (BMI) between 19 and 23  $\text{kg}/\text{m}^2$  at the first physical examination and between 18 and 24  $\text{kg}/\text{m}^2$  at the postal re-approach ( $n = 1732$ ). The second group had a BMI between 27 and 39  $\text{kg}/\text{m}^2$  and between 26 and 40  $\text{kg}/\text{m}^2$  at the examination and postal re-approach respectively ( $n = 1952$ ). None of the subjects had diagnosed diabetes mellitus. Buffy coats of all 3684 subjects, stored for 2–12 years, could be used to determine the IRS-1 *Gly972Arg* polymorphism. From this group, all carriers ( $n = 519$ ) and a random sample of wildtype subjects ( $n = 497$ ) were invited for a second physical examination. Sixty percent of the invitees participated. After the re-approach, 10 subjects had become diabetic and were excluded. Thus, data of 600 subjects could be used for the present analysis. Power analysis showed that this study size

has sufficient power (80% at a  $P$ -value of 5%) to detect a difference in fasting insulin of 10  $\text{pmol}/\text{l}$  (S.D. 30  $\text{pmol}/\text{l}$ ) between carriers and non-carriers among one of the weight groups, a difference for example observed by Baroni et al. [5] among lean subjects.

Subjects filled in an informed consent form before the measurements started. The protocol was approved by the Medical Ethical Committee of TNO Prevention and Health, Leiden, The Netherlands.

### 2.2. Clinical measurements

After an overnight fast, subjects were weighed without shoes to the nearest 0.1 kg. Waist and hip circumferences were measured to the nearest 0.1 cm and height was measured without shoes to the nearest 0.1 cm. Blood was drawn into a 10 ml vacuum tube containing  $\text{K}_3\text{EDTA}$  and a 4.5 ml vacuum tube containing 9 M sodium citrate for measurements to be reported elsewhere.

The subjects filled in a general health questionnaire, which included questions about cigarette smoking, alcohol consumption and physical activity. Subjects were classified as cigarette smokers or non-smokers. Alcohol consumption was categorised as follows: no alcohol, between 0 and 1 glass of alcohol a day, between 1 and 3 glasses of alcohol a day, and more than three glasses of alcohol a day. Bouts of exercise, biking or gardening etc. were included to assess physical activity if they lasted 30 min per day or more. Subjects who reported such activities on 3 or more days a week were considered to be physically active. Subjects active on 0–2 days a week were classified as non-active.

### 2.3. Laboratory measurements

DNA was extracted from buffy coats by proteinase K digestion and phenol extraction [13]. The *Gly972Arg* polymorphism was determined using the conventional polymerase chain reaction (PCR), *Bst*NI restriction enzyme analysis and agarose gel electrophoresis as described by Almind [4]. EDTA-plasma was used to measure total cholesterol and triglyceride, and HDL cholesterol concentrations after a dextran sulfate- $\text{Mg}^{2+}$  precipitation by enzymatic methods (Boehringer Mannheim, Germany). If triglyceride concentrations were below 4.5  $\text{mmol}/\text{l}$  LDL cholesterol was calculated by using the Friedewald equation [14]. Plasma glucose concentrations were measured with a commercially available kit (Roche Diagnostica, Basel, Switzerland and WAKO, Neuss, Germany). Insulin concentrations were determined with a sensitive ELISA kit with cross-reactivity with C-peptide and proinsulin less than 0.01% (Mercodia, Uppsala, Sweden). Proinsulin was also measured with an ELISA kit (Mercodia, Uppsala, Sweden). Insulin resistance was calculated with a homeostasis model assessment for insulin resistance ( $\text{HOMA}_{\text{IR}}$ ) as described previously [15].

## 2.4. Statistical analysis

A Kolmogorov–Smirnov test was performed to test normality of the data. Triglyceride, insulin, proinsulin and glucose concentrations and HOMA<sub>IR</sub> were not normally distributed, but were after a log transformation. Partial correlation coefficients between variables were calculated adjusted for age and sex. Differences in subject characteristics between the *Gly* and *Arg*-variant were examined by analysis of covariance with adjustments for age and sex. To examine the interaction between BMI-group (high or low) with genotype on the metabolic parameters, additional adjustments were made for waist-to-hip ratio, BMI, alcohol consumption, physical activity, and cigarette smoking. The interaction term was omitted from the statistical model if it did not reach statistical significance. In this way, the effects of the *Gly972Arg* polymorphism could be estimated. Data are expressed as mean  $\pm$  S.D. or median with inter-quartile range for skewed variables. A two-tailed value of  $P < 0.05$  was considered to be statistically significant. All analyses were performed using SPSS; PC release 10.0.

## 3. Results

Among the population-based sample of 3684 subjects the prevalence of the heterozygous and homozygous forms of the *Gly972Arg* polymorphism were 14.1 and 0.5%. The allele frequencies were not different between the overweight and non-overweight group (0.075 versus 0.076). The distribution of the genotypes was in Hardy–Weinberg equilibrium ( $P = 0.75$ ), both in the low and in the high BMI-group. Participants of the re-examination for metabolic risk markers (273 men, 327 women) were between 26 and 71 years old. Mean BMI was 21.9 kg/m<sup>2</sup> (S.D. 1.4 kg/m<sup>2</sup>) in the low BMI-group and 31.2 kg/m<sup>2</sup> (S.D. 3.0 kg/m<sup>2</sup>) in the high BMI-group. Due to the deliberate oversampling

of the carriers, this final study population included 362 non-carriers, 228 heterozygous carriers and 10 homozygous carriers of the *Gly972Arg* polymorphism.

The overweight group was older and included more men compared with the low BMI-group (Table 1). Comparisons were therefore made after adjustment for age and sex. After this adjustment, carriers in the high BMI-group had a significantly lower BMI compared with non-carriers. In the low BMI-group, however, there was no difference in BMI between carriers and non-carriers. A similar difference between carriers and non-carriers according to BMI was observed for waist-to-hip ratio ( $P < 0.001$ ). For all other parameters, no significant interaction between the *IRS-1 Gly972Arg* polymorphism and BMI was observed ( $P > 0.15$ ).

After adjustments for age, sex, BMI, waist-to-hip ratio, physical activity, alcohol consumption, and cigarette smoking, insulin concentrations were higher in carriers of the *Arg*-variant compared with the non-carriers (Table 2). The difference between carriers and non-carriers in the high BMI-group, however, was more pronounced than that in the low BMI-group (6.90 pmol/l or 12% versus 2.21 pmol/l or 7%; Table 3). Insulin resistance as estimated with the HOMA<sub>IR</sub> model was also significantly different between carriers and non-carriers ( $P = 0.029$ , Table 2). Glucose and proinsulin concentrations did not differ between carriers and non-carriers of the *Gly972Arg* polymorphism. Triglyceride concentrations were significantly lower in the *Arg*-variant compared with the *Gly*-variant (0.13 mmol/l or 11%,  $P = 0.001$ , Table 4). Again, differences were more pronounced in the high BMI-group (Table 5). Total cholesterol, LDL cholesterol and HDL cholesterol levels were not significantly different between both groups.

Correlation coefficients of insulin and HOMA<sub>IR</sub> with other metabolic parameters are listed in Table 6. Correlations did not differ between the high and low BMI-group. Triglyceride levels were inversely associated with HDL cholesterol ( $r = -0.40$ ,  $P < 0.001$ ).

Table 1  
Subject characteristics according to BMI-group and *Gly972Arg* polymorphism

	High BMI-group <sup>a</sup>			Low BMI-group		
	Wildtype (n = 180)	Arg-variant (n = 124)	Adjusted P-value <sup>b</sup>	Wildtype (n = 182)	Arg-variant (n = 114)	Adjusted P-value
Men (%)	59.4 <sup>c</sup>	52.4	0.145	38.5	27.2	0.040
Age (years)	56.4 $\pm$ 7.06	54.6 $\pm$ 9.94	0.062	49.3 $\pm$ 10.3	49.7 $\pm$ 10.6	0.645
Smoking (% yes)	17.2	16.1	0.831	41.8	38.6	0.528
Alcohol (% yes)	72.8	74.2	0.711	70.9	71.9	0.748
Physical activity (% >2 days a week)	60.3	56.3	0.984	65.9	72.7	0.370
BMI (kg/m <sup>2</sup> )	31.6 $\pm$ 3.04	30.5 $\pm$ 2.78	<0.001	21.9 $\pm$ 1.37	21.8 $\pm$ 1.48	0.742
Waist-to-hip ratio	0.96 $\pm$ 0.08	0.91 $\pm$ 0.09	<0.001	0.81 $\pm$ 0.07	0.80 $\pm$ 0.07	0.223

<sup>a</sup> Subjects in the high BMI-group had a BMI between 26 and 40 kg/m<sup>2</sup>, subjects in the low BMI-group had a BMI between 18 and 24 kg/m<sup>2</sup> (see Section 2).

<sup>b</sup> P-values are adjusted for age and sex.

<sup>c</sup> Values are means  $\pm$  S.D. or percentages.

Table 2

Crude median values and adjusted<sup>a</sup> differences in insulin, proinsulin, glucose and HOMA<sub>IR</sub> according to *Gly972Arg* polymorphism in the total study population

Metabolic risk factors	Wildtype (n = 362)	Arg-variant (n = 238)	Adjusted <i>P</i> -value
Insulin (pmol/l)	42.5 (27.8–60.8) <sup>b</sup>	44.8 (31.7–65.3)	
Adjusted difference	4.08 (0.55, 7.59) <sup>c</sup>		0.024
Proinsulin (pmol/l)	6.49 (4.09–12.0)	5.80 (3.95–10.8)	
Adjusted difference	−0.14 (−0.83, 0.58)		0.718
Glucose (mmol/l)	5.17 (4.84–5.63)	5.15 (4.77–5.60)	
Adjusted difference	−0.02 (−0.11, 0.08)		0.748
HOMA <sub>IR</sub>	1.57 (1.05–2.46)	1.70 (1.22–2.61)	
Adjusted difference	0.16 (0.017, 0.31)		0.029

<sup>a</sup> Differences and *P*-values are adjusted for age, sex, BMI, waist-to-hip ratio, smoking, physical activity and alcohol consumption by analysis of covariance.

<sup>b</sup> Values are means with S.D. or medians with inter-quartile ranges for skewed variables.

<sup>c</sup> Values are adjusted differences with 95% confidence intervals.

Table 3

Crude median values and adjusted<sup>a</sup> differences in insulin, proinsulin, glucose and according to *Gly972Arg* polymorphism HOMA<sub>IR</sub> among the high and low BMI-group

Metabolic risk factors	High BMI-group <sup>b</sup>			Low BMI-group		
	Wildtype (n = 180)	Arg-variant (n = 124)	Adjusted <i>P</i> -value	2Wildtype (n = 182)	Arg-variant (n = 114)	Adjusted <i>P</i> -value
Insulin (pmol/l)	57.5 (41.0–74.1) <sup>c</sup>	60.4 (42.3–79.7)		30.8 (21.9–44.1)	35.4 (22.2–46.2)	
Adjusted difference	6.90 (0.17, 13.6) <sup>d</sup>		0.044	2.21 (−1.78, 6.21)		0.279
Proinsulin (pmol/l)	10.9 (6.4–17.4)	9.09 (5.57–15.9)		4.52 (3.29–6.75)	4.30 (3.09–6.06)	
Adjusted difference	0.63 (−1.13, 2.39)		0.483	−0.30 (−0.93, 0.33)		0.345
Glucose (mmol/l)	5.40 (5.14–5.92)	5.41 (5.04–5.77)		4.93 (4.66–5.20)	4.85 (4.63–5.24)	
Adjusted difference	−0.02 (−0.21, 0.19)		0.835	−0.02 (−0.12, 0.09)		0.686
HOMA <sub>IR</sub>	2.34 (1.65–3.19)	2.33 (1.66–3.43)		1.19 (0.87–1.60)	1.32 (0.82–1.70)	
Adjusted difference	0.31 (0.01, 0.61)		0.042	0.07 (−0.07, 0.21)		0.327

<sup>a</sup> Differences and *P*-values are adjusted for age, sex, BMI, waist-to-hip ratio, smoking, physical activity and alcohol consumption by analysis of covariance.

<sup>b</sup> Subjects in the high BMI-group had a BMI between 26 and 40 kg/m<sup>2</sup>, subjects in the low BMI-group had a BMI between 18 and 24 kg/m<sup>2</sup> (see Section 2).

<sup>c</sup> Values are means with S.D. or medians with inter-quartile ranges for skewed variables.

<sup>d</sup> Values are adjusted differences with 95% confidence intervals.

Table 4

Crude means and adjusted<sup>a</sup> differences in serum lipids according to *Gly972Arg* polymorphism in the total study population

Metabolic risk factors	Wildtype (n = 362)	Arg-variant (n = 238)	Adjusted <i>P</i> -value
Total cholesterol (mmol/l)	5.36 ± 1.02 <sup>b</sup>	5.35 ± 1.00	
Adjusted difference	0.04 (−0.13, 0.20) <sup>c</sup>		0.660
LDL cholesterol (mmol/l)	3.48 ± 0.98	3.56 ± 0.93	
Adjusted difference	0.13 (−0.03, 0.29)		0.119
HDL cholesterol (mmol/l)	1.27 ± 0.36	1.26 ± 0.37	
Adjusted difference	−0.05 (−0.09, 0.003)		0.066
Triglyceride (mmol/l)	1.15 (0.75–1.71)	0.98 (0.63–1.52)	
Adjusted difference	−0.13 (−0.21, −0.05)		0.001

<sup>a</sup> Differences and *P*-values are adjusted for age, sex, BMI, waist-to-hip ratio, smoking, physical activity and alcohol consumption by analysis of covariance.

<sup>b</sup> Values are means with S.D. or medians with inter-quartile ranges for skewed variables.

<sup>c</sup> Values are adjusted differences with 95% confidence intervals.

Table 5

Crude values and adjusted<sup>a</sup> differences in serum lipids according to *Gly972Arg* polymorphism among the high and low BMI-group

Metabolic risk factors	High BMI-group <sup>b</sup>			Low BMI-group		
	Wildtype (n = 180)	Arg-variant (n = 124)	Adjusted P-value	Wildtype (n = 182)	Arg-variant (n = 114)	Adjusted P-value
Total cholesterol (mmol/l)	5.52 ± 0.98 <sup>c</sup>	5.49 ± 0.96		5.21 ± 1.04	5.20 ± 1.02	
Adjusted difference	0.01 (−0.24, 0.26) <sup>d</sup>		0.941	−0.04 (−0.26, 0.19)		0.754
LDL cholesterol (mmol/l)	3.65 ± 0.94	3.74 ± 0.89		3.31 ± 0.99	3.37 ± 0.94	
Adjusted difference	0.09 (−0.15, 0.34)		0.443	0.04 (−0.18, 0.26)		0.730
HDL cholesterol (mmol/l)	1.11 ± 0.25	1.12 ± 0.28		1.44 ± 0.38	1.41 ± 0.40	
Adjusted difference	−0.01 (−0.07, 0.05)		0.713	−0.06 (−0.14, 0.03)		0.175
Triglyceride (mmol/l)	1.54 (1.04–2.15)	1.20 (0.82–1.74)		0.91 (0.65–1.25)	0.74 (0.50–1.16)	
Adjusted difference	−0.26 (−0.46, −0.06)		0.010	−0.12 (−0.22, −0.01)		0.026

<sup>a</sup> Differences and P-values are adjusted for age, sex, BMI, waist-to-hip ratio, smoking, physical activity and alcohol consumption by analysis of covariance.<sup>b</sup> Subjects in the high BMI-group had a BMI between 26 and 40 kg/m<sup>2</sup>, subjects in the low BMI-group had a BMI between 18 and 24 kg/m<sup>2</sup> (see Section 2).<sup>c</sup> Values are means with S.D. or medians with inter-quartile ranges for skewed variables.<sup>d</sup> Values are adjusted differences with 95% confidence intervals.

Table 6

Partial<sup>a</sup> correlation coefficients between fasting insulin and HOMA<sub>IR</sub> and other metabolic parameters in the total study population

	Fasting insulin		HOMA <sub>IR</sub>	
	Partial r	P-value	Partial r	P-value
Glucose	0.36	<0.001	0.52	<0.001
Proinsulin	0.66	<0.001	0.71	<0.001
Triglycerides	0.31	<0.001	0.34	<0.001
Total cholesterol	0.02	0.548	0.03	0.468
LDL cholesterol	0.08	0.042	0.09	0.034
HDL cholesterol	−0.34	<0.001	−0.35	<0.001

<sup>a</sup> Results are adjusted for age and sex.

#### 4. Discussion

Previous studies on the impact of the codon 972Arg-variant of the IRS-1 gene on metabolic risk factors included a limited number of carriers, which may partly explain inconsistencies in the results. Using oversampling of the carriers within a population-based cohort we showed that the Arg-variant is associated with higher insulin concentrations and insulin resistance as assessed with the HOMA-model and lower triglyceride concentrations.

An effect of the *Gly972Arg* polymorphism of the IRS-1 gene on metabolic risk markers seems plausible, because of the central role of IRS-1 in the insulin signalling cascade. In support, Almind et al. [16] found that insulin signalling of myeloid progenitor cells transfected with the 972Arg-variant of the human IRS-1 was impaired as compared to cells transfected with wildtype human IRS-1. We recently showed, based on a meta-analysis of 3408 cases and 5419 controls from 27 studies, that the 972Arg-variant is associated with a small but significant increased risk of type 2 diabetes [3]. Human studies on metabolic factors, however, are less conclusive [4,5,7,9,17] which may be due to the limited num-

ber of carriers included. Further, previous studies have suggested that effects of this polymorphism may depend on BMI [5,7] with obese carriers, e.g. having up to 81.7 pmol/l higher fasting insulin levels than obese non-carriers [5].

Using oversampling of the carriers within a population-based cohort we showed that differences in fasting insulin and HOMA<sub>IR</sub> between carriers and non-carriers were more pronounced and statistically significant in the high BMI-group, but not in the low BMI-group. However, the differences were smaller than previously suggested. In addition, despite our large number of carriers the interaction term testing for modification by overweight was not statistically significant ( $P = 0.17$ ), indicating that the combined impact is not very strong.

Our results suggest that the IRS-1 *Gly972Arg* polymorphism is associated with insulin resistance, albeit to modest extent, whereas lower triglyceride levels were seen. Insulin resistance is generally associated with increased triglyceride concentrations [18], and our finding of lower triglyceride concentrations in carriers of the Arg-variant is therefore somewhat surprising and may be a chance finding. A similar finding has been reported before from type 2 diabetic patients and subjects with CHD [9]. This suggests that the observation in our population may not be due to chance only, and that exclusion of diabetic subjects from our study may not solely explain our results. We can only speculate about a further explanation. The insulin signalling pathway is involved in the insulin-dependent inhibition of hepatic VLDL-triglyceride production [19], but may in the liver involve IRS-2 rather than IRS-1 [2,20]. Therefore, in IRS-1 972Arg carriers, high insulin levels combined with relative hepatic insulin sensitivity could have resulted in reduced fasting triglycerides. This, however, remains to be confirmed by other studies.

Apart from affecting insulin sensitivity, also beta-cell function may be impaired in the IRS-1 *Gly972Arg*

polymorphism. Recently, human pancreatic islets from 972Arg carriers were found to have reduced insulin content, altered insulin release, and a greater number of immature secretory granules [21]. However, results of clinical studies so far are mixed [22,23]. We measured proinsulin as an indicator of a defect in insulin secretion [24]. Although proinsulin levels were strongly associated with insulin levels and HOMA<sub>IR</sub> in our population, they were not different between carriers and non-carriers of the Arg-variant.

Although fasting insulin and HOMA<sub>IR</sub> were inversely associated with HDL cholesterol in our population and reduced HDL cholesterol levels are a feature of insulin resistance [18], we observed no clear difference in HDL cholesterol between carriers and non-carriers. Insulin resistance is in general not uniformly associated with total cholesterol levels [25]. Indeed, HOMA<sub>IR</sub> and fasting insulin were not associated with total cholesterol levels in our population. In addition, total cholesterol concentrations were not different between carriers and non-carriers of the 972Arg-variant. This is in agreement with several other studies [5,7], although also higher [6] or lower [9] cholesterol levels have been reported in studies with smaller number of carriers. LDL particle size may change as a consequence of insulin resistance [26], but has not yet been studied in relation to the 972Arg-variant, nor has apolipoprotein B.

BMI was 1.2 kg/m<sup>2</sup> lower in our carriers than in non-carriers within the overweight group, and also their waist-to-hip ratio was reduced. These differences were not observed in the low BMI-group. A lower BMI and waist-to-hip ratio were also seen in healthy normal glucose tolerant 972Arg carriers from Germany [27]. Others observed a positive association between the IRS-1 972A variant and body weight [28]. Similarly, in type 2 diabetic patients an increased BMI in carriers was seen [17]. However, their control subjects over 40 years of age with the 972Arg-variant had in contrast a lower BMI compared with non-carriers.

The lower BMI in our overweight carriers might have been the result from the exclusion of diabetic subjects, as overweight carriers are more prone to develop type 2 diabetes. On the other hand, IRS-1 protein expression is markedly increased during maturation of human pre-adipocytes and IRS-1 is also relevant in IGF-1 signalling [2]. Our findings suggest therefore that studies on the impact of the 972Arg-variant on these processes are useful as well.

Finally, the prevalence of the Gly972Arg polymorphism among the population-based sample of our cohort was 14.6%. It should be noted that regional variance in The Netherlands has been reported (11–18%), but this was based on small sample sizes only and may be a chance finding [29]. Our proportion of carriers fell well within the range of the two previous observations and agrees with results of two other population-based studies in The Netherlands (15.3 and 14.1%) (R.M. van Dam, personal communication).

This large population-based study of healthy individuals shows that presence of the IRS-1 Gly972Arg polymorphism is associated with increased insulin concentrations and in-

sulin resistance and decreased triglyceride concentrations. These associations are more pronounced among overweight subjects, but the combined effect may be smaller than suggested previously.

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